Pre-Clinical Study of General Toxicity of the Medication "Fitin-S"

Kamola Mirzaakhmedova[1], Shakhida Ziyayeva[1], Abdushukiir Yuniisov[1], Gidchekhra Karimova[1], Rustam Karimov[1]

[1] Associate Professora. Tashkent Pediatric Medical Institute, Uzbekistan, Tashkent

Abstract

A pre-clinical study of the general toxicity of the medication "Fitin-S" has shown that it is referred to class IV of low toxic compounds. The medication "Fitin-S" does not have aaccumulative and local irritant action. At multiple intramuscular administration to rats and rabbits it does not influence the behavior and the dynamics of animals growth, does not cause a toxic effect on the composition of peripheral blood, kidneys' and liver's function as well as on the pathomorphology of animals organs and tissues. There is a slowdown in the process of blood coagulation in rabbits at doses of 16 and 32 mg/kg during the whole study period. In 1 month of a recovery period all the indicators have been changing within physiological norm. All the aforementioned data allows us to make a conclusion that the medication does not cause a toxic effect on the organism of animals.

Keywords: *General toxicity, medication "Fitin-S", acute toxicity, cumulation, chronic toxicity, local irritant action, pathomorphological study.*

Introduction

The purpose of studying the medication "Fitin-S" is to establish the nature and severity of its damaging effects on the body of experimental animals and assess its safety.

Materials and Method

The study of acute toxicity of the medicationshas been carried out according to the method of Litchfield and Wilcoxon on white mice, both sexes, weighing 20±2.0 g of 6 animals in each group, 150 mice has been used in total[2]. All pharmacological studies have been performed on healthy sexually mature animals (mice) exposed to the quarantine of at least 10-14 days[1].

The medication tested has been injected intravenously, subcutaneously and intramuscularly as a single dose of 100 to 10000 mg/kg. The animals were monitored hourly during the first day of the experiment in the laboratory, while the survival rate during the experiment, general condition, possible convulsions and death were used as indicators of the functional state of the animals. At the end of the experiment, the average lethal dose (LD50) has been calculated and the toxicity class has been determined[3]. The study of accumulation of "Fitin-S" has been carried out according to the Lim's method, which allows to evaluate not only accumulation, but also addiction. The objective of the study was to identify a possible accumulative property in the solution of the medication tested. The trials have been performed on 10 mice of both sexes weighing 20±2.0 g. The medications have been administered intramuscularly according to the scheme for 24 days from 0.1 to 1.15 from LD_{50}[4].

The general toxic action of "Fitin-S" at multiple intramuscular administration has been studied in 40 non-linear white rats (males and females) with the initial weight of 110±10 grams and in 16 gray-colored rabbits of both sexes with the initial weight 2,0±0,2 kilograms. Animals were in vivarium on a normal diet. The trial animals have been divided into 4 groups: 10 rats (5 males and 5 females) and 4 rabbits (2 females and 2 males) in each. The medication has been administered intramuscularly daily (7 days a week) for 30 days to rats at doses of 20, 40 and 100 mg/kg and to rabbits at 8.0, 16.0 and 40 mg/kg. The control animals have been

injected with distilled water in a bioequivalent volume. All animals have been kept in standard vivarium conditions on a full-fledged food and water diet.

The condition of animals during chronic intramuscular administration has been evaluated by the following parameters of peripheral blood: haemoglobin contents, gr/dl; the number of red blood cells,10^{12}/l; reticulocytes,‰; thrombocytes, 10^9/l; leucocytes, 10^9/ land leukogram; liver

function evaluation - by glucose content,mmol/1; total protein,gr/dl; alanine - and aspartate - aminotransferases (A1AT, AsAT) in blood serum. Biochemical tests have been performed using test kits from CypressDiagnostica, Belgium. Renal function has beenevaluated by dimesis with a water load (5% of body weight) for 4 hours, blood urea, urine acidity (pH), the presence of nitrites, glucose, leukocytes etc. in the urine (qualitative reactions) with the Combina kit, Germany, and by urinary sediment.

Hematological and biochemical studies were performed after 10 and 30 days post the day of the experiment. At that time animals were weighed. At the end of the experiment some animals have been decapitated and the material has been taken for pathomorphological studies according to the list approved by Pharmacological Committee of the Republic of Uzbekistan[5], The rest of animals has been left to study the recovery process.

The general direction of changes in the coagulation process under the influence of medication has been assessed by the records of thromboelastograms performed on the thromboelastograph Tromb-2.

The "open field" test is a study of behavior due to the animal being in an unfamiliar open space, which it cannot leave. The animal is put in the central square, and the latent period of exit from it is recorder. The number of squares into which the animal entered is registered (horizontal motion activity - HMA), as well as the number of rises on the hind limbs ("upright posture" - VMA), the number of holes the animal snifled(exploratory activity - EA), the number of washes (number of grooming acts -NGA) and defecations (number of bolus - NB). Based on these parameters, motion activity, orientative-motion activity, anxiety and autonomic reactions are evaluated. The above parameters are fixed for 4 minutes from the moment of placing the animal[7].

The conjunctival sample is a very sensitive test and, in some cases, even discloses the reaction of animals to an allergen at mild allergization and negative skin tests.

The trials have been performed on 12 rabbits, weighing 2,0-2,5 kg, which have been instilled with 0,1 ml of 0,5 and 5% medication solution in the left eye, and with 0,1 ml of distilled water in the right eye (a controlled one). The reaction has been recorded in 15 minutes (quick reaction) and in 24-48 horns (delayed hypersensitivity) and evaluated according to the following scale (in points). Besides, the level of hyperemia, swelling, and lacrimation have beenregistered[8].

The local-irritant action of "Fitin-S" has been studied on 12 rats weighed 150±10 grams. The animals have had their hair clipped on both sides of the spinal column (4 areas) 2x2 cm in size.0,5 ml of Fitin-S in 0,5 and 5% solutions have been applied on two clipped areas on the left side of the back. The medication has been applied for 10 days. The controlled areas were the right clipped areas to which distilled water has been applied in the same volume. The monitoring has been carried out for 14 days. The skin reaction has been recorded daily according to the skin sample scale in points[8].

Statistical processing of the obtained data has been carried out with the definition of the Student's criterion using statistical programs WindowsExcel 2010.

All experimental animals have been kept in the same conditions and on a common diet with free access to water and food.

Results

The general action and "acute" toxicity of "Fitin-S" have been determined in mice at a single intravenous, intramuscular and subcutaneous administration. Each dose of the substance has been studied in 6 animals. The monitoring has been conducted for 14 days.

The medication has been administered to mice intravenously at doses of 200,300,400, 500 and 600 mg/ kg in the form of a 0.5% solution, intramuscularly and subcutaneously at doses of 100-500-1000-2500-2700- 2800-2900-3000-3300-3600 mg/kg as a 5% solution.

When studying the acute toxicity of the medication "Fitin-S" with intravenous administration, a 5% solution of the medication has been diluted with 0.9% sodium chloride in a ratio of 1: 1; 1: 5 and 1:10. The obtained solutions of the "Fitin-S" medication have been administered intravenously at doses from 100 to 400 mg/kg in a volume not exceeding 0.5 ml per animal weight[3]. As can be seen from the data in table 1, after

administration of the medication at doses causing toxic phenomena, convulsions and death of some mice have been observed within 72 hours. The average lethal dose (LD50) of intravenous administration of the "Fitin-S" medication to mice has amounted to 347 (320 +- 460) mg/kg.

The results of studies with subcutaneous and intramuscular administration have shown that "Fitin-S" at doses of 100-1500 mg/kg does not cause changes in the general condition of the animals. When the medication is administered at doses of 1800-2500 mg/ kg, after 30-60 minutes there has been a decrease in the motion activity of animals, while the response of mice to external stimuli has remained unchanged, and no lethal outcome has been observed. With the introduction of the medication at doses of 2800-3600 mg/kg after 60-120 minutes, anxiety of mice, shortness of breath, short-term convulsions and the death of some animals during the day has been registered.

Table 1: The results of indicators of "acute" toxicity during parenteral administration of the medication "Fitin-S" to mice

Animal species, type of administration	Sex	Dose mg/kg	Number of animals in a group/number of animals died	LD_{10} -m+m mg/kg	LD_{16} -m+m mg/kg	LD_{50} -m+m mg/kg	LD_{S4} -m+m mg/kg
Mice, intravenous	Males	150	6/0	233 -4,8 +6,0	294 -6,0 +7,6	347 -9,3 +13,1	460 -9,7 +12,3
		200	6/2				
		250	6/4				
		300	6/5				
		400	6/6				
Mice, intramuscular	Males	1800	6/0	2000 -380 +450	2266 -300 +330	2560 -350 +380	2893 -500 +550
		2000	6/0				
		2200	6/2				
		2500	6/3				
		2800	6/5				
		3000	6/6				
Mice, subcutaneous		2200	6/0	2240 -300 +330	2566 -320 +420	2900 -340 +380	3277 -440 +480
		2500	6/1				
		2900	6/3				
		3000	6/4				
		3300	6/6				
		3600	6/6				

Discussion

Thus, the study of the acute toxicity of "Fitin-S" has shown that the medication is referred to class IV of low toxic compounds. The LD_{50} during intravenous, intramuscular and subcutaneous administration to mice has amounted to 347(320+460) mg/kg respectively, 2560 (2266+2893) mg/kg and 2900(2566-3277) mg/kg.

When choosing the doses for the study of chronic toxicity of pharmacological substances their accumulative effect should be taken into account. That's why before conducting experiments on chronic toxicity we have been studying the accumulation via subchronic toxicity according to the method of Lim and coauthors which allows to evaluate not only accumulative properties but also addiction, and we have determined

 the accumulation index, the ratio of LD50 with a single administration to LD50 with a multiple administration.

The studies conducted have shown that the accumulation index K_K has amounted to$K_K=LD_{50n}/ LD_{50}$ 2944/2560=1,15. In other words, the medication "Fitin-S" does not have the accumulative effect, the accumulation rate is 1,15.

The local-irritant effect of the medication has been studied on rats and rabbits. The results of monitoring have shown that "Fitin-S" in 5 and 10% concentrations does not cause even the slight reddening of the conjunctiva of the rabbit eye in either 15 minutes or 24 or 48 hours. Application of the medication for 10 days on the skin of rats also does not cause irritation, redness, swelling or other visible changes on the skin and the effect of "Fitin-S" is estimated at 0 points.

According to this a conclusion that the medication "Fitin-S" in 5 and 10% concentrations does not have the irritant effect on the conjunctiva of rabbits and on the skin of rats can be made.

The results obtained at daily intramuscular administration of "Fitin-S" to rats and rabbits are represented in Tables 3-4. As can be seen from the data given in tables 3-4, in 10 and 30 administration at all the studied doses there has been no significant deviations in the composition of the peripheral blood of rats and rabbits in the hemoglobin content of erythrocytes, in the number of blood cells themselves, their precursors (reticulocytes), and leucocytes. Leukocyte counts in rats and rabbits are within the physiological norm.

Based on this, it can be concluded that "Fitin-S" at the studied doses with intramuscular repeated administration does not have a toxic effect on the quantitative composition and morphology of the peripheral blood of experimental animals.

Table 2: Indicators of peripheral blood of rats at daily intramuscular administration of the medication "Fitin-S" (M±m; n=10)

Indicators	Control	10 days, dosemg/kg			30 days, dosemg/kg		
		20	40	100	20	40	100
Hemoglobin, gr/dl	14,7±0,3	15,8±0,7	15,9il,0	15,0±l,0	14,8i0,2	14,0i0,l	13,3i0,2
Erythrocytes, 10^{12}/l	5,2±0,5	5,8i0,5	5,0i0,6	5,8i0,4	5,H0,3	4,8i0,2	5,0i0,6
Reticulocytes, %	4,2±0,2	5,4i0,3	5,8i0,3	3,9i0,3	6,5i0,4	6,5i0,5	4,3i0,4
Thrombocytes, 10 ^{9}/l	675±11,5	830il2,5	600±ll,0	550il0,5	675il2	725il4	725il4
Leucocytes, 10^{9}/l	10,2±0,8	ll,6±0,8	12,2±0,9	13,3i0,7	8,4i0,6	9,1±1,2	ll,9±0,7
Neutrophils:							
Bands, %	l,8±0,2	2,0i0,2	2,0i0,2	2,0i0,2	2,0i0,2	2,0i0,2	2,0i0,2
Segmentonuclear, %	25,0±l,0	27,4i0,2	27,2i0,8	27,5il,0	28,5il,5	28,7il,3	28,5il,5
Eosinophils, %	2,0i0,2	2,0i0,2	2,0i0,2	2,0i0,2	2,0i0,3	2,0i0,2	2,0i0,2
Monocytes, %	3,0i0,3	2,6i0,2	2,9i0,3	3,0i0,3	3,0i0,3	3,0i0,3	3,0i0,3
Lymphocytes, %	64,2±1,8	66,0i3,4	66,8i3,2	65,5i3,5	65,Oil,6	64,3i2,7	64,5il,5

*P < 0,05 to control

Table 3: Indicators of peripheral blood of rabbits at daily intramuscular administration of the medication "Fitin-S" (M±m; n=4)

Indicators	Control	10 days, dosemg/kg			30 days, dosemg/kg		
		8	16	40	8	16	40
Hemoglobin, r/dl	9,6±0,3	9,9i0,7	12,0±0,5	H,2i0,l	9,2i0,7	8,4i0,5	8,4±0,l
Erythrocytes, 10 ^{12}/1	4,5±0,5	4,HO,5	5,0i0,6	4,7i0,8	4,3i0,5	4,4i0,6	4,0i0,8
Reticulocytes, %	3,5±0,4	4,0i0,6	3,2i0,5	5,5i0,5	4,4i0,6	3,2i0,2	4,0i0,3
Thrombocytes, 10 ^{9}/l	350±ll,5	250i6,5	225il3,0	400i8,5	250i6,5	325il3,0	400i8,5
Leucocytes, 10^{9}/l	8,0±0,8	8,0i0,6	8,25i0,9	5,4i0,4*	9,4i0,6	9,0i0,9	8,4i0,4
Neutrophils:							
Bands, %	l,8±0,2	2,2i0,2	l,9±0,2	2,0i0,2	l,8i0,2	2,0i0,2	l,9±0,2
segmentonuclear, %	25,0±l,0	27,4i0,2	27,2i0,8	27,5±l,0	27,0i0,2	27,2i0,8	27,5±l,0
eosinophils, %	2,0i0,2	2,0i0,2	2,0i0,2	2,0i0,2	2,0i0,2	2,0i0,2	2,0i0,2
basophils, %	l,0±0,l	0,8i0,6	l,li0,l	l,0±0,l	l,2i0,l	l,li0,l	l,0±0,l
monocytes, %	3,0i0,3	2,6i0,2	2,9i0,3	2,8i0,3	2,6i0,2	2,8i0,3	2,9i0,3
lymphocytes, %	63,2±1,8	65,0i3,4	65,8i3,2	64,7i3,5	65,0i3,4	65,8i3,2	64,5i3,5

*P < 0,05 to control

Diuresis after 10 days and 30 days of the experiment under conditions of water load did not differ significantly in the experimental and control rats. In rats, the state of nitrogen metabolism, the criterion of which is the urea content in the blood serum, did not deviate from the control after 10 days and 1 month of the experiment. In the urine during the entire experiment, both in the experimental groups and in the control group, no changes in the studied urine parameters have been detected. The acidity of urine in all the studied groups has not been changing during the entire experiment and its pH was 6.0-7.0. No pathological changes in urine sediment have been detected.

The results obtained have led to the conclusion that the medication "Fitin-S" with chronic administration does not have a toxic effect on renal function in the studied doses.

The liver function in experiments on rabbits and rats has been studied with the use of the most sensitive tests. The levels of total protein, glucose, and enzyme activity (AsAT, A1AT) in the blood serum of all experimental rats after 10 and 30 days of the experiment have not differed from the control (Table 4.5).

Thus, the medication "Fitin-S" at intramuscular daily administration at the studied doses does not have a toxic effect on liver function.

Table 4: Some indicators of liver function of rats at intramuscular administration of the medication "Fitin-S" in a chronic trial (M±m; n=5)

Study timeline	Dose mg/kg	Total protein, gr/1	AlAt, mmol/l	AsAt, mmol/l	Glucosemmol/1	Urea mmol/l
10 days	Control	84i5,3	0,26i0,03	0,35i0,04	5, HO,4	4,4i0,42
	"Fitin-S" 20	78i5,2	0,28i0,02	0,35i0,03	5,62i0,4	4,6i0,4
	40	82i5,5	0,22i0,02	0,27i0,02	5,9i0,4	4,4i0,42
	100	82i5,2	0,2 HO,02	0,39i0,04	5,9i0,4	4,6i0,4
30 days	Control	72i5,6	0,35i0,03	0,40i0,03	5,6i0,4	4,2i0,4
	"Fitin-S" 20	86i5,6	0,3i0,02	0,36i0,03	5,5i0,4	4,3i0,4
	40	76i6,0	0,28i0,02	0,36i0,02	5,6i0,4	4,5i0,42
	100	76i6,0	0,28i0,02	0,4i0,03	5,5i0,4	4,6i0,4

P > 0,05 to control

Table 5: Some indicators of liver function of rabbits at intramuscular administration of the medication "Fitin-S" in a chronic trial (M±m; n=5)

Study timeline	Dose mg/kg	Total protein, gr/1	AlAt, mmol/l	AsAt, mmol/l	Glucosemmol/1	Urea mmol/l
10 days	Control	84±5,3	0,16±0,03	0,25±0,04	2,l±0,4	4,4±0,42
	"Fitin-S"-8	68±5,2	0,18±0,02	0,22±0,03	0,62±0,4	4,6±0,4
	16	82±5,5	0,18±0,02	0,27±0,02	l,9±0,4	4,4±0,42
	40	82±5,2	0,21±0,02	0,3±0,04	2,9±0,4	4,6±0,4
30 days	Control	72±5,6	0,25±0,03	0,32±0,03	5,6±0,4	4,2±0,4
	"Fitin-S"-8	86±5,6	0,3±0,02	042±0,03	5,5±0,4	4,3±0,4
	16	76±6,0	0,26±0,02	0,36±0,02	5,6±0,4	4,5±0,42
	40	76±6,0	0,28±0,02	0,34±0,03	5,5±0,4	4,6±0,4

P > 0,05 to control

As the medication "Fitin-S" is supposed to be applied parenterally, it was important to find out how the process of blood coagulation changes at multiple application. The experiments have been held on 12 chinchilla rabbits, males and females weighing 2,0±0,2 kg. The blood has been studied in 10 and 30 days post the administration of "Fitin-S" at doses of 8,0, 16,0 and 40,0 mg/kg.

The results of the impact of the medication "Fitin-S" on the process of blood coagulation are represented in table 6. Thromboelastogram data indicate that after 10 and 30 administrations, "Fitin-S" does not affect the blood coagulation process at a therapeutic dose of 8 mg/kg. With an increase in the therapeutic dose by 2 and 5 times (16 and 40 mg/kg), the blood coagulation process changes, which is expressed in an increase in the indicator K, the time of clot formation, which depends on the concentration of thrombin formed and the amount of fibrinogen; and a decrease in MA, a decrease in the elasticity of the clot (E) and the index of hypercoagulation Ci, which indicates a slight decrease in the process of blood coagulation.

Table 6: The dynamics of changes in blood coagulation of rabbits with chronic administration of the medication "Fitin-S" M±m; n=4)

TEG Indicators	Control	In 10 days/dosemg/kg			In 30 days/dose mg/kg		
		8,0	16,0	40,0	8,0	16,0	40,0
R, mm	50±2,l	40±2,0	45±l,0	50±l,0	40±2,0	45±l,0	50±l,0
K, mm	15±l,0	15±1,1	25±l,0	30±0,6*	15±1,1	25±l,0*	30±0,6*
R+K, mm	65±3,5	55±3,0	70±3,0	80±6,6	55±3,0	70±3,0	80±6,6
R/K	3,3±0,2	2,8±0,l	l,8±0,l	l,7±0,l*	2,8±0,l	l,8±0,l*	l,7±0,l*
MA, mm	69±1,5	66±l,0	64±l,0	62±1,4	66±l,0	64±l,0	62±1,4
t, mm	100±10	100±10	100±10	100±10	100±10	100±10	100±10
S, mm	115±11	115±10	125±11	130±12	115±10	125±11	130±12
T, mm	165±14	155±14	170±15	180±16	155±14	170±15	180±16
Ci (MA/R+K)	l,l±0,l	l,2±0,l	0,9±0,l	0,8±0,06	l,2±0,l	0,91±0,l	0,8±0,06
E, MAxlOO/lOO-MA	222±19	194±16	178±15	163±14	194±16	178±15,0	163±14
ITC (E/S)	l,9±0,l	l,7±0,l	l,4±0,l	l,26±0, *	l,7±0,l	l,4±0,l	l,26±0,l*

*P < 0,05 to control

Thus, the intramuscular administration of the medication “Fitin-S” to rabbits at a therapeutic dose of 8 mg/kg does not cause anyeffect, andat doses of 16.0 and 40.0 mg/kg leads to a slight slowdown in blood coagulation process throughout the entire period of administration (30 days).

The study of the effect of the medication “Fitin-S” on the central nervous system and peripheral nervous system in the open field test has shown that after 10 days of administration of “Fitin-S” for 4 minutes, it has not changed the horizontal and vertical motion activity of rats at doses of 20, 40 and 100 mg/kg. After 30 days of the experiment, a sedative effect has been observed, which was expressed in a reduction in the number of intersections (horizontal activity) and the number of postures (vertical activity) and the number of “holes” (exploratory activity). Throughout the entire experiment, "Fitin-S'lias not significantly affected the vegetative reactions of the body (the number of “boluses”, the number of washings).

Table 7: Impact of “Fitin-S” on behavioral reactions of animals in the “open field” test (M±m; n=6)

Medication, dose, mg/kg	Latentperiod, sec	Motion activity		Quantity		
		Horizontal, number oflntersections	Vertical, number ofpostures	Holes	Washes	Bolus
In 10 days						
Control	l,0±0,l	46,3±3,4	5,3i0,4	9,7±l,0	l,0±0	l,0±0,l
“Fitin” C-20	0,5±002	38,0±2,6	5,5i0,4	8,0i0,4	l,3±0,l	l,8±0,15
40	0±0	43,0±2,4	5,5i0,4	10,8±0,8	l,0±0,l	l,3±0,ll
100	0,75±002	49,0±3,6	7,8i0,6	6,0i0,4*	0,3±0,01	0,4i0,04
In 30 days						
“Fitin” C-20	2,2±0,2	14,0±l,0*	2,5i0,2*	2,5i0,2*	0,45i00,2	l,0±0,3
40	3,8±0,2	32,3i3,0	3,6i0,2*	4,8i0,4*	0,6i0,04	l,4i0,l
100	l,0±0,l	26,0i2,3*	2,0i0,2*	5,0i0,4	l,3±0,l	0,5i0,02

*P < 0,05 to control

The study of integral indicators has shown that the dynamics of the body weight of the experimental animals at the studied doses after 10 and 30 days of the experiment has not differed from the control. Throughout the experiment animals were active, neat, they ate food normally, drank water, their hair was smooth, shiny. The behavior of experimental rats has not differed from the behavior of control groups of animals.

A macroscopic examination of sacrificed animals has disclosed the correct location of the internal organs. There is no free fluid in the pleural and abdominal cavity. Tissues of the lungs, stomach and intestines are also of a normal color, without signs of edema, hemorrhages and ulcerations. Pancreas, kidneys and adrenal glands are unchanged.

At the end of the experiment, 1 cm^3 of organs has been taken from experimental animals for histological examination. This site has been fixed in 10% normal formalin. Then the tissue has been embedded in paraffin and sections of histological preparations 3-5 microns thick have been prepared and stained with a mixture of hematoxylin + eosin. The obtained preparations have been analyzed under a Leica-1000 DM microscope (Germany), IOx eyepiece, lOx and 40xlens [6]

The results of histological studies have shown that basically their histostructure is comparable with the morphology of control groups of animals. In the stomach, the mucous membrane is well preserved, glandular cells are without any changes. In the small intestine its main elementsare villi and crypts. They are well prominent, villi are finger-shaped, the border of enterocytes is distinguished, the stroma of the villi and the epithelial layer are moderately saturated with lymphocytes, are distinctly distinguished in the enterocytes. In the colon, the crypts are lined with mucous cells, the lumens of the crypts are enlarged and filled with mucoid secretion.

In the liver of experimental animals treated with the medication, no significant changes have been found. In experimental animals at the studied doses, the majority of hepatocytes are in a state of hydropic edema with granular inclusions, and moderately medium-droplet fat inclusions on the periphery. In addition, small foci of cellular infiltration have beendisclosed in the liver parenchyma in some areas.

Basically, as usual, the boundaries of the lobules are not distinct in the liver, the hepatocytes are polygonal in shape, their cytoplasm is pink and contains a large, roundish one or two nuclei. In stellate macrophages, small grains of hemosiderin have also been found, as well as in the control. The structure of the various departments of the nephron, renal corpuscles, proximal and distal parts are without any changes. The structure of the capsule and cellular elements of all departments of the nephron indicates their functional viability.

White and red pulp are seen in the spleen; the structure of these elements does not differ significantly from that of control animals. The study of the structural organization of the myocardium is indistinguishable from indicators in the control group, consists of rectangular cardiomyocytes, with a weakly pronounced transverse striation.

The lungs have a characteristic pattern and are represented by numerous dilated alveolar vesicles and atelectasis sites. The interalveolar walls contain numerous blood capillaries, the walls of the bronchi and blood vessels are without any significant changes.

The histology of the thymus is represented by the cortical and brain zones, with signs of age-related involution. The cortical zone is represented by lymphoid cells and trabecular interlayers. Also, the brain area is represented by lymphoid follicles, without clear centers of reproduction.

In this regard, it should be noted that the differences found are a manifestation of the species or age characteristics of animals.

Thus, the results of histological studies allow us to conclude that “Fitin-S” at the studied doses is the optimal medication, its long-term use does not cause noticeable structural destruction or damage and can be recommended for further clinical trials.

Conclusion

Pre-clinical study of the general toxicity of the medication “Fitin-S” has shown that it is referred to class IV of low toxic compounds. The medication “Fitin-S” does not have anaccumulative and local-irritant effect. With repeated intramuscular administration to rats and rabbits, it does not affect the behavior and dynamics of animal weight, does not have a toxic effect on the composition of peripheral blood, kidney and liver function, and also on the pathomorphology of animal organs and tissues. With repeated use, there is a slight slowdown in the blood coagulation process in rabbits at doses of 16 and 32 mg/kg throughout the study period. After 1 month of the recovery period, all these indicators have been changing within the physiological norm. All of the above data allow us to conclude that the medication does not have a toxic effect on the animal organism.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

References

1. M.L. B. Elements for qualitative evaluation of the pharmacological effect [Element! kolichestvennoi otsenki farmakologicheskogo effekta]. L. «Medgiz». 1963.
2. T.A G. Toxicology of medications [Toksikologiya lekarstvennykh sredstv]. Moscow. 2008;: p. 27-30.
3. A.V. Stefanov. K. Pre-clinical study of medications (guidelines) [Doklinicheskoye issledovaniye lekarstvennykh sredstv (metodicheskiye rekomendatsii)]. 2002;: p. 587.
4. A.N. M. Guidelines for the pre-clinical study of the general toxic effects ofmedications [Metodicheskiye rekomendatsii po doklinicheskomu izucheniyu obshetoksicheskogo deistviya lekarstvennykh sredstv]/Guidelines for pre-clinical studies of medications. Parton. 2012;: p. 13-23.
5. Instructions for preclinical safety testing of pharmacological agents [Instrukcii po doklinicheskomu ispitaniyu bezopasnosti farmakologicheskikh sredstv]. Tashkent. 2000.
6. others BVPa. Laboratory method for studying the hemostasis system [Laboratomiye metody issledovaniya sistemy gemostaza]. Tomsk. 1980.
7. Markel G.A. KRA. The method of complex registration of behavioral and autonomic reactions in rats during the open “field test” [Metod kompleksnoi registratsii povedencheskikh I vegetativnykh reaktsiy u kris pri provedenii testa otkritoye pole]. Zhum.vish. nervn. deyat. 1976; 6(26): p. 1314-1318.
8. Meditsina ebDSSaYLPM. Preparation of histological sections. (Microscopic Technique: Manual) [Prigotovleniye gistologicheskikh srezov. (Mikroslopicheskaya tekhnika: Rukovodstvo)]. 1996; p. 432.
9. Mirzaahmedova, K. T., Abdullaeva, S. K., Akhmadiev, E. E., & Ziyayeva, S. H. (2017). The effect of immunomoduline and phytin compounds on lipid peroxidation induced at toxic experimental

hepatitis. In *International Conference on Chemical, Biological and Health Sciences* (pp. 99-106).

10. Калдыбаева, А. О., and А. А. Абдусаматов. "Влияние корня солодки, цветков бессмертника и мумиё на показатели перекисного окисления липидов при хроническом токсическом гепатите." *Медицинский журнал Узбекистана* 3 (2010): 99-101.
11. Мирзаахмедова, К. Т., А. А. Абдусаматов, and А. Н. Набиев. "Влияние иммуномодулина и соединений фитина на показатели перекисного окисления липидов при экспериментальном токсическом гепатите." *Сибирский медицинский журнал (Иркутск)* 88.5 (2009): 122-124.
12. Aminov, S. D., and A. A. Vakhabov. "Pharmacology of some coumarins isolated from plant Haplophyllum." *Dokl. Acad. Nauk. UzSSR* 8 (1985): 44-45.
13. Аминов, С. Д., and К. Т. Мирзаахмедова. "Изучение гастропротекторных свойств глицитрината на экспериментальных животных." *Педиатрия* 1-2 (2013): 118.